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STUDIES ON RESOLUTION OF RACEMIC GOSSYPOL

——SEPARATION OF HEXAACETATES OF *S*-1-METHYLPHENETHYLAMINO DERIVATIVE OF (±) GOSSYPOL*

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ABSTRACT

This paper reports the resolution of racemic gossypol to optically active enantiomery by condensation of (±) gossypol with *S*-1-methylphenethylamine, followed by subsequent acetylation and hydrolysis. Seven isomers of hexaacetate of di-S-1-methylphenet-thylamino gossypol have been obtained by chromatography on silica gel, and also structures of the acetates have been determined.

Racemic gossypol (I), a polyhydroxydinaphthaldehyde present in the seed, root and stem of cotton (*Gossypium*), has been proved to be a potential male antifertility agent[1]. The (+) gossypol isolated from *Thespesia populnea* was reported to be lacking in antifertility activity[2]. This result led one to postulate that (−) gossypol might be the biologically active enantiomer. In recent years, scientists in China and abroad as well have paid much attention to the study on the resolution of the racemic compound and the search for the natural source of (−) enantiomer.

The resolution of racemic gossypol was attempted by Dechary et al.[3] in 1971. In their report, with optically active alkaloids such as morphine, quinine, cinchonine they tried to resolve gossypol through the salt formation of the acidic phenolic gossypol and they also tried to resolve gossypol through condensation of various optically active primary amines, such as *S*-1-methylphenethylamine, *R*-1-naphthylethylamine and *S*-2-amino-1-propanol with the aldehydic group in gossypol. But neither method gave satisfactory result. No experimental details were given in their paper. Thereafter many laboratories had been involved in the study on the resolution, and negative results obtained urged one to suspect the optical instability of gossypol. However, by reviewing the properties of (+) gossypol, reasonable optical stability of (+) gossypol was revealed. We therefore concluded that under suitable conditions (±) gossypol can be resolved. In 1983, a communication[4] of the resolution of racemic gossypol was reported by our Laboratory, and Matlin[5] reported the resolution by HPLC in 1984.

S-1-Methylphenethylamine was first selecte as resolving agent. The yellow crystalline condenasation product of (±) gossypol with *S*-1-methylphenthylamine give m.p. 197—200°C, $[\alpha]_D^{36} = +223.6$ and m/z 753 $(M+1)^+$ indicating two moles of amine being involved. It ex-

* A part of Si Yikang's thesis toward her M. S. degree.

Table 1

Specific Rotations, Melting Points and Crucial Characteristic Peaks in ^1H NMR

Hexaacetate	$[\alpha]_D$(c,$CHCl_3$)	m.p.(℃)	^1H NMR			
			C_4-H	C_4-H	C_{11}H	C_{11}-H
A	577.8(0.194)		7.87	7.30	8.48	7.44
B	959.6(0.114)	208—210	7.82	7.82	8.56	8.56
C	−99(0.157)	212—214	7.38	7.38	7.50	7.50
D	−343.7(0.135)	155—156	7.90	7.26	8.45	7.48
E	475.6(0.186)	145—147	7.92	subsided in benzene peak	8.70	7.50
F	−554.8(0.110)	211—213	7.73	7.73	8.60	8.60
G	−192.4(0.092)	150—153	7.88	7.28	8.68	7.52

Table 2

IR and UV Data of Hexaacetates

Hexaacetate	IR(cm^{-1})		UV nm (logε)
	$-\overset{O}{\overset{\|}{C}}-O$	$-\overset{O}{\overset{\|}{C}}-N-$	
A	1770	1670	212(4.69),249(4.78),307(4.27),332(4.09),400(3.98),424(3.98).
B	1760	—	206(4.77),242(4.89),305(4.39),342(4.23),402(4.36),424(4.36).
C	1760	1670	214(4.88),254(4.97),317(4.11),332(4.07).
D	1770	1680	212(4.76),249(4.87),307(4.31),332(4.20),400(4.11),424(4.11).
E	1765	1670	212(4.72),249(4.85),307(4.27),332(4.15),400(4.08),424(4.08).
F[a]	1760	—	205(4.83),243(4.94),305(4.40),342(4.27),402;
G	1770	1680	212(4.74),249(4.87),307(4.32),332(4.19),400(4.12),424(4.12).

a) Range of measurement: 205—400 nm.

hibited two yellow spots on TLC with R_f values of 0.62 and 0.54 respectively. Separation by fractional crystallization using various solvents was unsuccessful. Crystals obtained from various fractions showed no improvement of specific rotation and gave two yellow spots on TLC with the same R_f values as the starting material. Equilibration between two spots was observed on two-dimensional TLC.

In order to avoid the unexpected change, looking for hydroxy groups by acetylation was carried out. Acetylation of condensation mixture of (±) gossypol with *S*-1-methylphenethylamine in pyridine by acetic anhydride afforded a yellowish powder. On TLC (SiO_2 plate, developed by hexane: ethyl acetate 1:1) it exhibited five green fluorescent spots with R_f values of 0.55, 0.52, 0.48, 0.36 and 0.33 under ultraviolet light. By repeated chromatotron or column chromatography followed by recrystallization, seven components A, B, C, E, F and G were obtained. Among them, B, C and D gave the same R_f value of 0.52 on TLC under the condition mentioned above. The same molecular peak m/z 1004 $(M)^+$ or 1005 $(M+1)^+$ in mass spectra of the seven compounds was observed, which coincided with the molecular weight of hexaacetates. Their specific rotations, melting points, crucial characteristic peaks in NMR and IR and UV maximum adsorption peaks are listed in Tables 1 and 2.

Compounds B and F, unlike the others, gave no amide carbonyl peak at 1680 cm^{-1} in IR.

Since simpler spectra were observed in their ^1H NMR spectra, a symmetrical molecule composed of two identical halves was suggested. Compound B gave two singlets located (in the lower field) at δ 8.56 and 7.82 and two corresponding peaks at δ 8.60 and 7.73 were observed in the ^1H NMR spectra of component F. The singlets at δ 8.56 (in B) and 8.60 (in F) were assigned to the protons of Schiff base —N=C_{11}-H and the singlets at δ 7.82 and 7.73 were ascribed to C_4 protons para to the —OAc at C_1 of the naphthalene ring. It was proposed that B and F formed a pair of diastereoisomers, i.e. hexaacetates of *S*-amino-(−)-gossypol and *S*-amino-(+)-gossypol of Structure II (aldehpde form).

Compound C showed an amide band at 1670 cm^{-1} in IR spectra and a simpler ^1H NMR spectrum with no peak at field lower than δ 8.0 was observed. Instead, two singlets corresponding to two protons appeared at δ 7.50 and 7.38. The peak at δ 7.38 was assigned to C_4-H by NOE, whose intensity increased by 23% on irradiating the peak at δ 2.41 of C_8 methyl, and the peak of (δ) 7.50 should belong to the C_{11}H of cyclic acetal. A symmetrical molecule with Structure III was proposed for component C.

The IR spectra of components A, D, E and G all showed an absorption band at 1670 cm^{-1} or 1680 cm^{-1}. It explained the presence of an amide-carbonyl group. In their ^1H NMR spectra, one proton singlet, appearing at δ 8.48, 8.45, 8.70 and 8.68 respectively, was ascribed to C_{11}H of Schiff base moiety (—CH=NR structure) and signal of C_4 proton para to C_1-OAc was reasonably located at δ 7.84—7.92. Another two singlets for each compound were found at the regions of δ 7.50 and 7.30 (δ 7.44, 7.30 for A, 7.48, 7.26 for D, 7.50, 7.20 for E and 7.52, 7.28 for G). One of them in the region of δ 7.50 should belong to C_{11}'-H of the cyclic acetal form and the peaks at δ 7.30 region were assigned to C_4'-H as in compound C. It is reasonable to find higher field peak for proton C_4'-H para to C_1' O-alkyl than to the -OAc attached to C_1. The structures of A, D, E and G were therefore proposed to consist of one Schiff base and one cyclic acetal group (Structure IV).

The seven hexaacetates gave 3 types of UV spectra corresponding to the bis-Schiff base structure II (compounds B and F), the biscyclic acetal structure III (compound C) and unsymmetrical structure IV (compounds A, D, E, G). In the bis-Schiff base, the short wavelength peaks were located at 206 and 243 nm, while in the diacetal form C, the corresponding shifts of ca. 8 and 10 nm to large wavelength were observed. The 305 and 342 nm peaks of the former structure shift to 317 and 332 nm correspondingly in the latter structure. In the spectrum of the diacetal compound C, no peaks were observed in the region > 400 nm while the bis-Schiff base gave two peaks at 402 and 424 nm. Probably this set of absorption might arise from the extension of conjugation in Schiff base. The compounds A, D, E and G of unsymmetrical structure showed absorption peaks of the two chromophores in Structures II and III.

Thus, from the acetylation of (±) gossypol amino condensation product, two Schiff bases, one with cyclic acetal structure and four unsymmetrical molecules with Schiff base structure and cyclic acetal structure in the same molecule were obtained. Four diastereoisomers, A, D, E and G were isolated instead of the expected two, this probably was due to the possible *cis-trans* of C_{11}H of the Schiff base or the chirality of the chiral C_{11}H created in cyclic acetal formation. Theoretically, if the *cis-trans* of Schiff base and (*R*)-(*S*) of the C_{11}H were considered along with chirality of binaphthyl group, then 32 diastereoisomers of hexaacetates should be isolated. Apparently, the effects of the atropisomerism and the structure isomerism (Schiff base and cyclic acetal) were the dominant factors affecting the separation by chromatography.

Therefore only seven isomers were isolated. Besides, the total amount of the unsymmetrical compounds obtained was larger than that of symmetrical Schiff base or diacetal compound. This can be explained by the higher probability of forming this unsymmetrical structure.

Hydrolysis of compound A (+[α]) and compound D (−[α]) gave (+) and (−) gossypols correspondingly. After recrystallization, they gave identical melting point (166—167°C). The retention time on HPLC, UV, ^{1}H NMR and mass spectra were also identical with each other and were the same as that of racemic gossypol. But their specific rotations were opposite in sign, i.e. $[\alpha]_D$ +359 (c, 0.053, $CHCl_3$)[1] and $[\alpha]_D$ −353.2 (c, 0.056, $CHCl_3$). Their CD curves were in mirror image to each other (Fig. 1). Among the remaining hexaacetates, the compound with (+) rotation gave (+) gossypol and that with (−) rotation gave (−) gossypol.

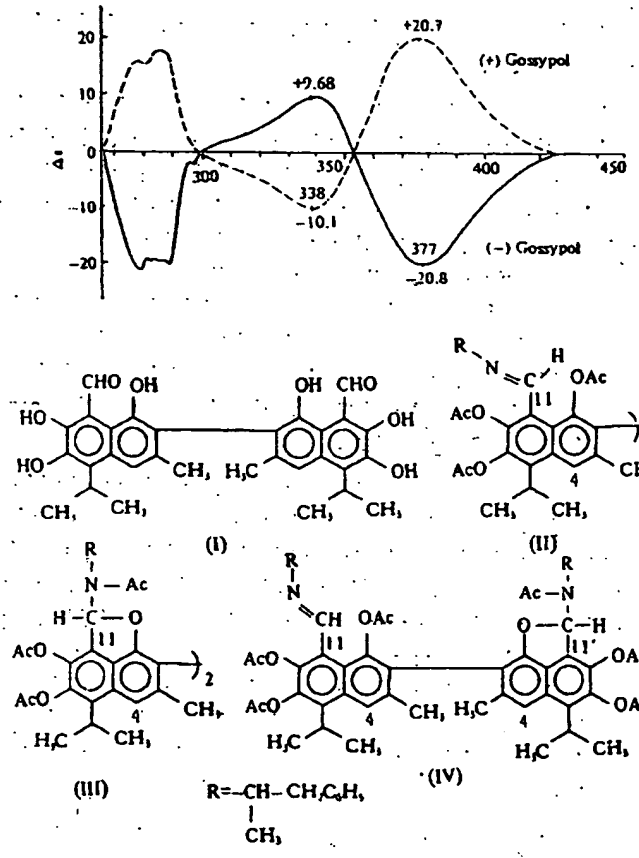

Fig. 1

The labile property of the condensation product of gossypol and chiral amine was preventted by acetylation, through which the first successful resolution of (±) gossypol was given. However, the tautomerism during acetylation gave a complicate mixture of hexaacetates and

1) This data was identical with that of natural (+) gossypol provided by Prof. Fang of Illinois University, that is, $[\alpha]_D$ + 340° ($CHCl_3$), m. p. 167—168°C, but lower than the previously reported $[\alpha]_D$ + 445° ($CHCl_3$)[4].

brought on the tedious separation, so an improved method for practical preparation has been developed. In a recent communication[7] the keto-enamine structure of the condensation product of amine and gossypol, and the direct separation of distereoisonmers of the condensation product have been reported by our laboratory.

As (+) gossypol is inactive in the antifertility test in rats, the Pharmacological Department of our Institute[8] has proved that (−) gossypol is the active isomer. But the result[9] of similar inhibitory activities of the three isomers, (+), (−), (±) gossypols, *in vitro* on LDH-X which has been proposed[10a] to be the target enzyme for the (±) gossypol antifertility action makes the proposal questionable.

Experimental

Melting points were determined on a Reichert melting point microscopic apparatus (uncorrected). Perkin-Elmer 399 and 683 spectrophotometers (IR spectra), Shimadzu UV 240, 300 and 360 spectrophotometers (UV spectra), WH-90, Jeol FX-90Q and Jeol FX-100Q spectrometers (NMR spectra), ZAB-2F, MAT-731 spectrometers (mass spectra) were used for spectra determination. Polarimeter Perkin-Elmer 241 was used for optical rotation and circular dichroism curve was performed on Johin-Yvon Mark V. Treatment of silica gel used: commerical silica gel was washed subsequently several times with concentrated HCl (chemically pure), distilled water and 95% ethanol and then dried at 120°C for 8 h.

1. *Preparation of di-S-1-methylphenethylamino gossypol*

Five g (0.0096 mole) of (±) gossypol was dissolved in 400 ml isopropyl alcohol under refluxing. The solution was cooled and 2.86 g (0.021 mole) of *S*-1-methylphenethylamine ($[\alpha]_D^{30} = +35.7$, neat, $d = 0.949$, $l = 1$ dm), was added. After refluxing for 5 min, the solution was concentrated and golden yellow solid separated out. After being filtered and washed with isopropyl alcohol, 6.95 g (yield 95.7%) of solid was obtained and then recrystallized from anhydrous ethyl alcohol. The melting point was 197—200°C, $[\alpha]_D^{24} = +223.6$ (c, 0.55, $CHCl_3$) and two yellow spots were exhibited, $R_f = 0.62$ and 0.54 on TLC (silica gel plate, developed by anhydrous ether: petroleum ether = 1:1).

Analysis for $C_{48}H_{52}N_2O_6$

Calcd. (%): C, 76.57; H, 6.96; N, 3.73.

Found (%): C, 76.64; H, 7.00, N, 3.58.

UV (95% ethanol) nm (log ε): 207 (4.62), 246 (4.89), 270 (shoulder, 4.64), 300 (shoulder, 4.22), 402 (4.33).

IR (KBr) cm^{-1}: 3480—3280, 3020, 2960, 2920, 2870, 1620 (strong), 1610 (strong), 1530, 1450, 1380, 1240, 1130, 840, 740, 700.

^1H NMR (90 MHz, $CDCl_3$) δ: 1.40 (2 × 3H, d, $J = 7$ Hz, 2 $-CHCH_3$), 1.53 (4 × 3H, d, $J = 7$ Hz, 2 $(CH_3)_2CH-$), 2.09(2 × 3H, s, 2 CH_3Ar-), 2.93 (2 × 2H, d, $J = 6$Hz, 2 $-CH_2Ar$), 3.71 (4 × 1H, m, 2—HC(CH_3)$_2$ and 2 —HC—N—), 7.18 (2 × 5H, m, 2 C_6H_5-), 7.54(2 × 1H, s, 2 C_4H), 9.47(2 × 1H, d, $2C_{11}H$), 5.38(2 × 1H, s, —OH), 8.0(2 × 1H, br., —OH), 13.50(2 × 1H, br., —NH).

MS, m/z: 753(M^++1, 9), 618(17), 617(39), 590(19), 527(30), 525(25), 510(22), 498(67), 496(39), 482(100).

2. *Preparation of Hexaacetate of di-S-1-methylphenethylamino gossypol*

Acetic anhydride (5 ml) was added to a solution of 0.5 g di-*S*-1-methylphenethylamino gossypol in 10 ml dry pyridine. The mixture was heated on a boiling water bath for 50 min. After the addition of 10 ml acetic acid dropwise to the cooled solution (exothermic), it was poured gradually into cold water with stirring. Pale yellow solid precipitated immediately and was filtered, and washed thorougly with distilled water to remove pyridine. The solid obtained wighed 0.526 g (yield 78.8%). It exhibited five bright green spots (R_f 0.55, 0.52, 0.48, 0.36, 0.33) on silica TLC developed by hexane: ethyl acetate = 1:1, under ultraviolet at 254 nm.

3. *Separation and Purification of Hexaacetates*

The above hexaacetates were chromatographed on silica (200—300 sieves) column with hexane: ethyl acetate (2:1) as eluant. From various fractions of elute, compound A, mixture of B, C and D, compound E and mixture of F and G were obtained. Mixture B, C and D was further separated on a silica column and eluted with toluene: butanone (8:2) to give D and mixture of B and C, with R_f 0.19 and 0.23 repectively on TLC system (silica gel, developed by toluene: butanone = 8.2). Mixture of F and G was separated into F and G under the above conditions.

Solids D, E, F, G and mixture of B and C were crystallized separately from a mixed solvent, hexane-ethyl acetate or benzene-cyclohexane. Compound B was precipitated in a crystalline form from a solution of mixture of B and C, while C was left in the mother liquid. After concentration of the mother liquid. C precipitated out. All these compounds were recrystallized three times or more until their specific rotation remained constant. Compound A was obtained in foamy solids on evaporating the eluate from repeated chromatotron.

4. *Hydrolysis of Hexaacetates*

One part of compound D was dissolved in 20 parts of peroxide-free THF in the presence of 8 drops of 9 *N* HCl and hydrolyzed at 40°C for 10—15 days in the dark under nitrogen, monitored by TLC. At the end of hydrolysis, THF was taken off under reduced pressure. The residue was dissolved in peroxide-free ether, washed with 0.2% sodium bisulfide solution, dried and evaporated under reduced pressure. The residue was separated by TLC (on treated silica gel, developed by anhydrous ether: petroleum ether (30—60°C) = 1:1). The bright yellow band was eluted by peroxide-free anhydrous ether and the solvent was removed under nitrogen. After recrystallization from benzene, yellow needles were obtained. A single peak with retention time identical with racemic gossypol was observed on HPLC. The crystal gave $[\alpha]_D^{23}$ = −353.2 (C, 0.056, $CHCl_3$) and m.p. 166—167°C.

UV (95% ethanol), nm (log ε): 234(4.82), 276(4.40), 380(4.19).

IR (film) cm^{-1}: 3510, 1620, 1595, 1570, 1430, 1415, 1380, 1285, 1240, 1190, 1120, 830.

^1H NMR (90 MHz, $CDCl_3$) δ: 1.55(d, J = 6 Hz, 12H, —CH(CH_3)$_2$), 2.15(s, 6H, Ar—CH_3), 3.86(m, 2H, Ar—CH(CH_3)$_2$), 5.78 (s, 2H, —OH), 6.40(s, 2H, —OH),

7.78(2H, C_4, $C_{4'}$, —H), 11.13(s, 2H, —C(=O)—H), 15.17(s, 2H, —OH).

MS, m/z: 518(M^+,6), 500(39), 482(100), 467(50), 454(11), 439(13), 226(9), 205(7), 150(9), 149(80).

CD: $\Delta\varepsilon_{377} = -20.8$; $\Delta_{338} = +9.68$(c, 1.19 mg/ml, $CHCl_3$).

When the same procedure was used to hydrolyze compound A, (+) gossypol was obtained. $[\alpha]_D = +359.0$ (c, 0.0054, $CHCl_3$), m. p. 165—166℃.

IR: the same as (—)gossypol.

CD: $\Delta_{377} = +20.7$, $\Delta_{338} = -10.1$ (c, 1.075 mg/ml, $CHCl_3$)

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